NCATS
COLLABORATE. INNOVATE. ACCELERATE.

The Pre-clinical (and Clinical) Utilities of Microphysiological Systems as In Vitro NAMs in Drug and Vaccine Development

Animal testing replacement for vaccines A One Health View: global outlook and future strategy
December 4, 2025 (virtual)

Danilo A.Tagle, PhD, MS
Director, Office of Special Initiatives
National Center for Advancing Translational Sciences

No Conflicts of Interest

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The Public Health Challenge

10,000

Diseases

and only

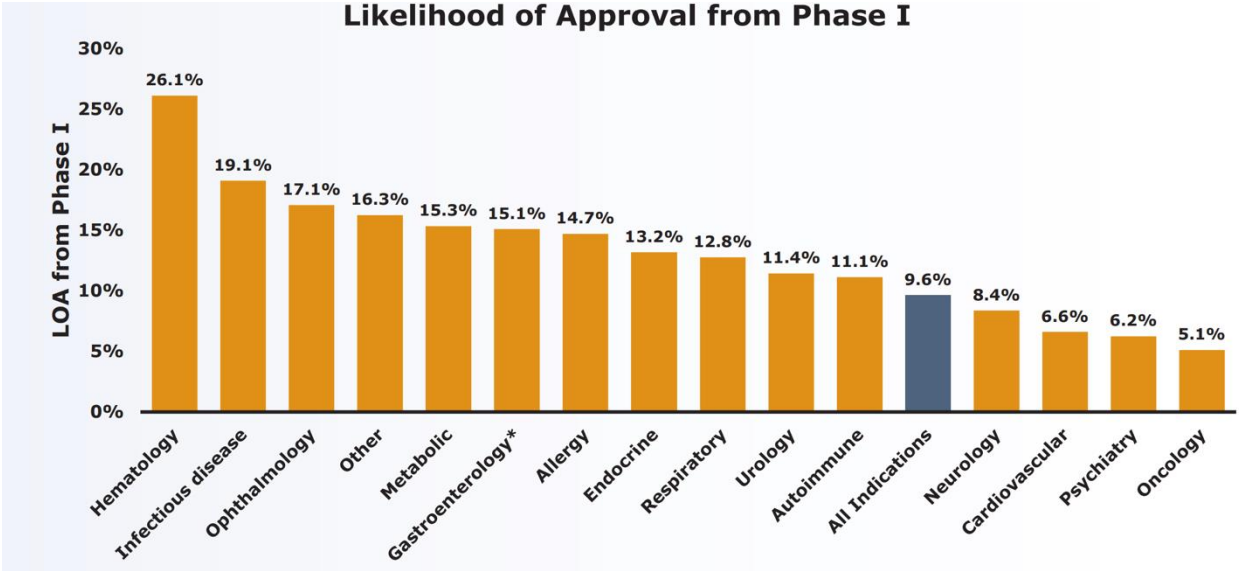
5%

Have Treatments or Cures

Time from early development to the medicine cabinet takes 10-15 years at a cost of $2.6 billion per drug ($6.16 billion in the past 20 years)

9 out of 10

HIGH ATTRITION RATE -
Promising therapeutic candidates often fail in clinical trials



Need for human-relevant model systems that can reliably predict health outcomes that can vary due to sex, age, genetics, population variability and interindividual differences

Arrowsmith and Miller, Nature Reviews Drug Discovery, 2013,, 12: 569 ;Cook et al., Nature Reviews Drug Discovery, 2014,13,: 19 ; Wong et al. Biostatistics, 2019, 20: 273-286

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NAMs (aka New Approach Methodologies, New Alternative Methods, Non-Animal Methods)

In Chemico

- Cell-free methods
- Epigenetics
- Biochemical pathways
- Chemical genetics
- Examples:
  - Synthetic biology
  - Gene circuits
  - Protein assays for irritancy

In Vitro

- Cultured cell methods
- Induced Pluripotent Stem Cells (iPSC)
- Microphysiological Systems
- Examples:
  - Tissue Chips
  - 3-D Tissue Bioprinted Constructs
  - Organoids and spheroids

In Silico

- Computational methods
- Artificial Intelligence, Large Language models, and Machine Learning
- Mathematical Modeling and Simulations
- Examples:
  - Virtual human
  - Digital Twins

New Approach Methodologies (NAMs)

The US regulatory and funding priorities surrounding the use of NAMs has evolved in recent months

FDA NEWS RELEASE

FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs

For Immediate Release: April 10, 2025

Today, the U.S. Food and Drug Administration is taking a groundbreaking step to advance public health by replacing animal testing in the development of monoclonal antibody therapies and other drugs with more effective, human-relevant methods. The new approach is designed to improve drug safety and accelerate the evaluation process, while reducing animal experimentation, lowering research and development (R&D) costs, and ultimately, drug prices.

NIH, FDA Announce New Joint Venture in Nutrition

The Food and Drug Administration (FDA) and NIH have announced a new research collaboration.

With diet-related chronic diseases continually rising, it's imperative that the FDA and NIH work closely to prioritize a better understanding of the root causes to end the diet-related chronic disease crisis and safeguard the health of America's children.

Under the new Nutrition Regulatory Science Program, the FDA and NIH will implement and accelerate a comprehensive nutrition research agenda that will inform effective food and nutrition policy actions to help make Americans' food and diets healthier.

NIH RECORD

June 6, 2025
Vol. LXXVII, No. 12

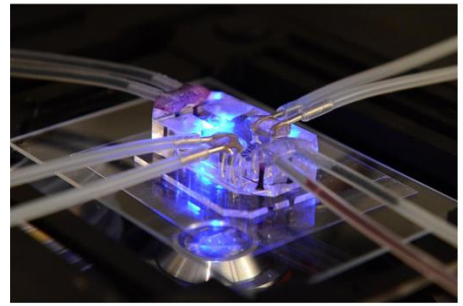
MPS for Chemical Risk Assessments in Food

Tuesday, April 29, 2025

NIH to prioritize human-based research technologies

New initiative aims to reduce use of animals in NIH-funded research.

The National Institutes of Health (NIH) is adopting a new initiative to expand innovative, human-based science while reducing animal use in research. Developing and using cutting-edge alternative nonanimal research models aligns with the U.S. Food and Drug Administration's (FDA) recent initiative to reduce testing in animals. While traditional animal models continue to be vital to advancing scientific knowledge, using new and emerging technologies can offer unique strengths that, when utilized correctly or in combination, can expand the toolbox for researchers to answer previously difficult or unanswerable biomedical research questions.

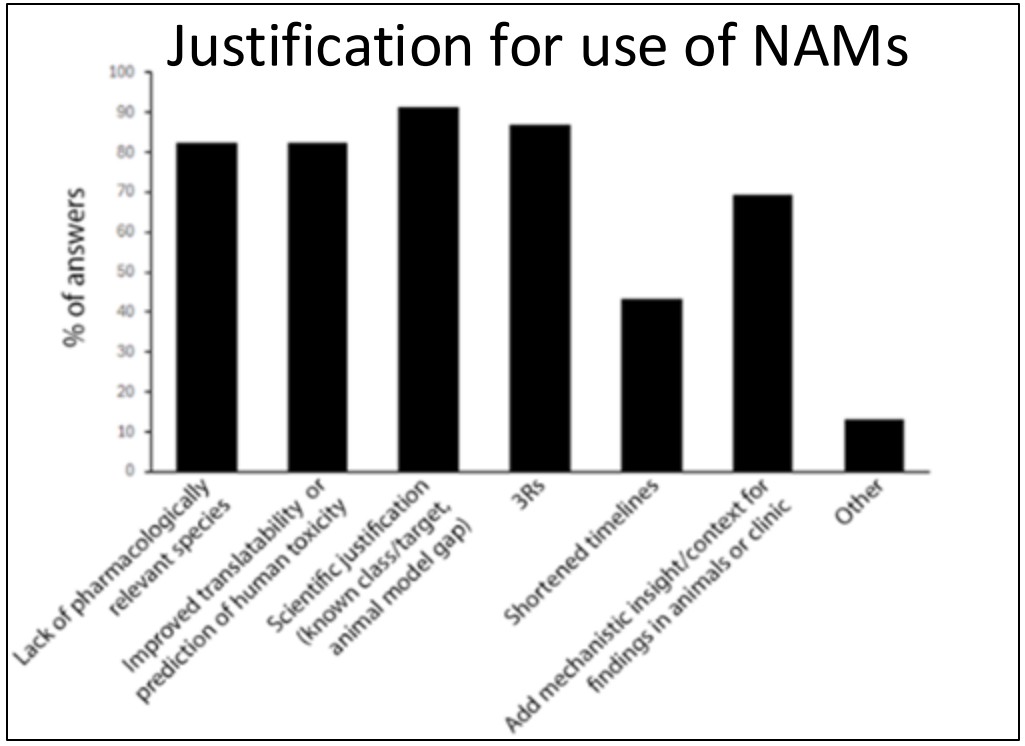


Zeldin to pursue new ban on animal testing at EPA

Administrator Lee Zeldin plans to revive a ban on animal testing at the Environmental Protection Agency, The Washington Times has learned.

The EPA had pursued a phaseout of animal testing during the first Trump administration, but the Biden administration erased the deadlines, effectively neutering the policy.

A Survey of Industry-wide Use of NAMs in Regulatory Filings



- Biotechnology Innovation Organization surveyed 27 companies about NAM usage and collected case studies showcasing NAM-based regulatory filings for biotherapeutics, where NAMs replaced large animal studies for safety assessment.
- Scientifically justified NAM-based regulatory submissions are accepted globally.
- Key justifications are no relevant species, prior experience with the target, and disease severity.
- Opportunity exists to scientifically justify NAM-based approaches in broader settings.
- Knowledge sharing on suitable scenarios for NAM-based filings is a general need.
- Updated regulatory guidance (e.g., ICH S6) would enable increased application and adoption.

Sharing NAMs Use Cases from Pharma Industries

nature reviews drug discovery

https://doi.org/10.1038/s41573-025-01182-9

Perspective

Check for updates

Application of new approach methodologies for nonclinical safety assessment of drug candidates

Mario Beilmann[1], Karissa Adkins[2], Harrie C. M. Boonen[3], Philip Hewitt[4], Wenyue Hu[5], Robert Mader[6], Susanne Moore[7], Payal Rana[8], Thomas Steger-Hartmann[9], Remi Villenave[6] & Terry van Vleet[10]

Categories:

1. Animal species lack the target
2. Cross-species target issues
3. Non-mammalian targets
4. Unpredicted clinical events

Category	Example	Drug candidate	Species used	Type of NAM applied	Key results	Outcome
1. Animal species lack the target	1	NBE: IMCgp100 (tebentafusp) BiTE	Human (in vitro)	2D human cancer cell lines and primary cells in co-cultures, complemented by in silico approach	Calculated MABEL and identified safe starting dose for FiH studies	Advanced into clinical development
	2	NBE: MEDI-565 BiTE	Human (in vitro)	Co-cultures of human peripheral blood mononuclear cells and CEA-positive human tumour target cells	Calculated MABEL and identified safe starting dose for phase I clinical studies	Advanced into clinical development
	3	NBE: EpCAM-targeted TCB and CEA-targeted TCBs	Human (in vitro)	Patient-derived intestinal organoids co-encapsulated with immune cells in hydrogel	Identified potential immune-related intestinal toxicities of drug candidates	Advanced into clinical development
2. Cross-species target issues	1	NCE: PDE3A–SLFN12 complex inducer BAY 2666605	Rats and NHPs (in vivo), human and NHPs (in vitro)	Human and NHP primary cells (aortic smooth muscle and endothelial cells)	Identified potential risks and safety margin	Advanced into clinical development
	2	NBE: anti-CD38 antibody SAR444559	NHPs (in vivo), human and NHP (in vitro)	NHP and human blood samples	Identified NHP-specific on-target finding	Advanced into clinical development
	3	NBE: trastuzumab and trastuzumab emtansine	Mice and rats (in vivo), human (in vitro)	Rodent in vivo studies and panel of HER2-positive and negative human tumour cell lines	Identified lack of cross-reactivity in rodent species and target-specific cytotoxicity	Advanced into clinical development
3. Non-mammalian targets	1	NCE: PeEF2 inhibitor M5717	Not applicable	In silico approaches including BLAST analyses	Identified selectivity for parasitic target	Advanced into clinical development
	2	Nucleoside analogues: AZT, fialuridine; nucleotide analogue: sofosbuvir	Various (in vivo), human (in vitro)	Comparison of in vivo studies and in vitro assays with relevant human cell lines	Identified off-target effects such as mitochondrial toxicity	Advanced into clinical development
4. Unpredicted clinical events	1	NCE: AKR1C3 inhibitor BAY 1128688	Rats and NHPs (in vivo), human (in vitro)	Repeat-dose toxicity studies, in vitro assays and DILIsym in silico modelling	Identified human-specific toxicity, most likely BSEP inhibition	Stopped owing to safety concerns
	2	NBE: humanized CD154-specific monoclonal antibody Hu5c8	Human (in vitro)	Microengineered model 'Vessel-Chip' composed of human endothelium and blood components	Revealed prothrombotic effect of Hu5c8	Identified potential risk for thrombosis
	3	Oligonucleotide: antisense oligonucleotide SPC5001	Human (in vitro)	Kidney proximal tubule-on-a-chip model	Induced cytotoxicity and increased levels of kidney injury biomarkers as indication of acute kidney injury observed in clinical but not in preclinical studies	Identified nephrotoxicity

AZT, azidothymidine; BiTE, bispecific T cell engager; BLAST, basic local alignment search tool; BSEP, bile salt export pump; CEA, carcinoembryonic antigen; DILI, drug-induced liver injury; EpCAM, epithelial cell adhesion molecule; FiH, first-in-human; HER2, human epidermal growth factor receptor 2; MABEL, minimum anticipated biological effect level; NAM, new approach methodology; NBE, new biologic entity; NCE, new chemical entity; NHP, non-human primate; TCB, T cell-engaging bispecific antibody.

https://www.nature.com/articles/s41573-025-01182-9

NAMs Safety Assessment Framework

1 - Biologics	2 - Biologics / CT	3 - Biologics	4 - Small molecules
High target specificity*, no cross-reactivity, no pharmacological relevant animal species -Oncology	**High target specificity**, cross-reactive and +/- pharmacological relevant animal species, clinically validated target & MoA	**High target specificity**, cross-reactive and pharmacological relevant animal species, new target/MoA	Often **lower target specificity than biologics**, pharmacology relevant animal species

Acceptance by health authorities

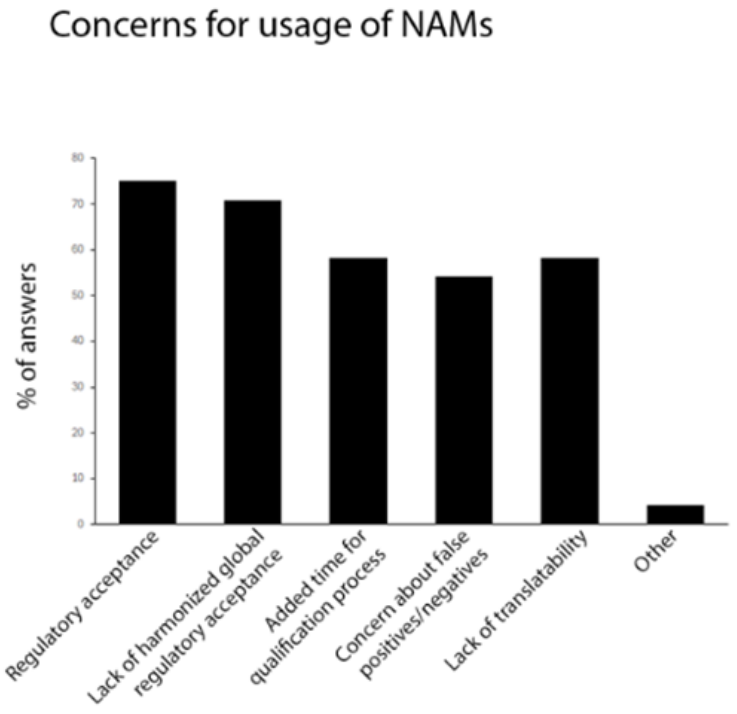
Challenge to replace animal models

Modalities	**Antibodies (**Multi-specific, T-cell engagers, co-stimulators)	**Cell therapy** (Engineered T cells)	**Antibodies** (Anti-amyloid)	**Antibodies** (Bispecific T-cell engager)	**Antibodies** (Anti-cytokine mAb (Fc-modified)	Hemophilia A therapy (?)	Rare diseases (?)
Indications	**Advanced cancer** (pMHC targets, solid tumors, hematology, ICH S9, DART)	**Advanced cancer**	**Serum amyloidosis** (SLTD, non-ICH S9)	**Virology** (SLTD, non-ICH S9)	**Respiratory disease** (Non-LTD, unmet need)	**Hemophilia A** (Non-LTD)	**Rare diseases** (SLTD, non-ICH S9)

Perceived Barriers to NAMs Usage

Despite the data suggesting that NAMs-based regulatory submissions were being accepted, most respondents had multiple concerns for usage of NAMs including:

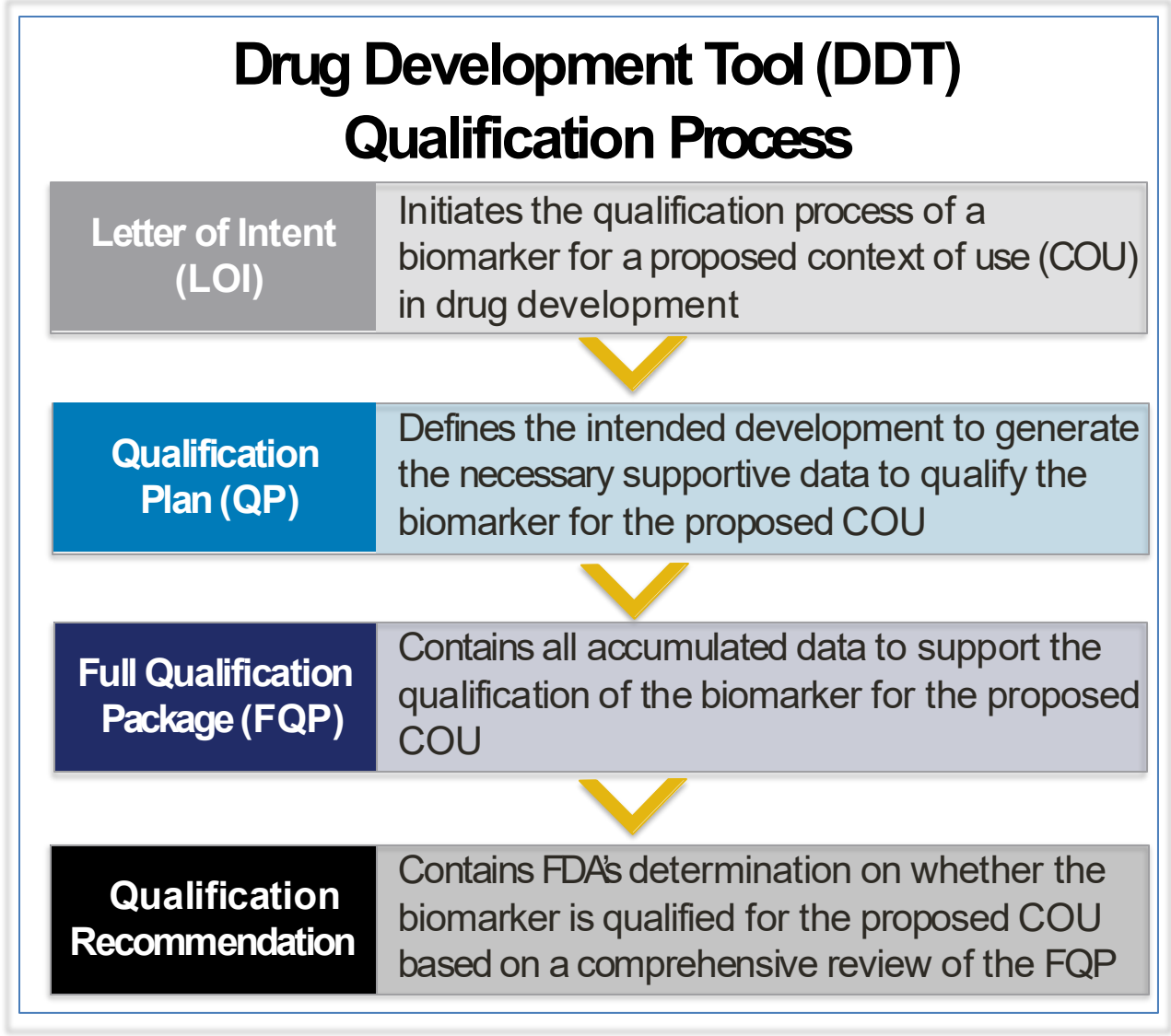
- Regulatory acceptance by FDA
- Lack of harmonized global regulatory acceptance
- Concerns about translatability
- Added time for the qualification/validation of novel NAMs
- Concern about false positive/negative



Regulatory Acceptance - FDA Qualification Process

Qualification - An assessment that a model or assay can be relied upon to have a specific interpretation and application in product development and regulatory decision making.

- Evaluates the fitness of the model for a **specific context of use**
- Identifies the boundaries of the available data that adequately justify the use of the tool.
- **Context of use (CoU)** - Delineates the manner and purpose for the method or approach as defined in the 1) Use statement and 2) Conditions for Qualified Use

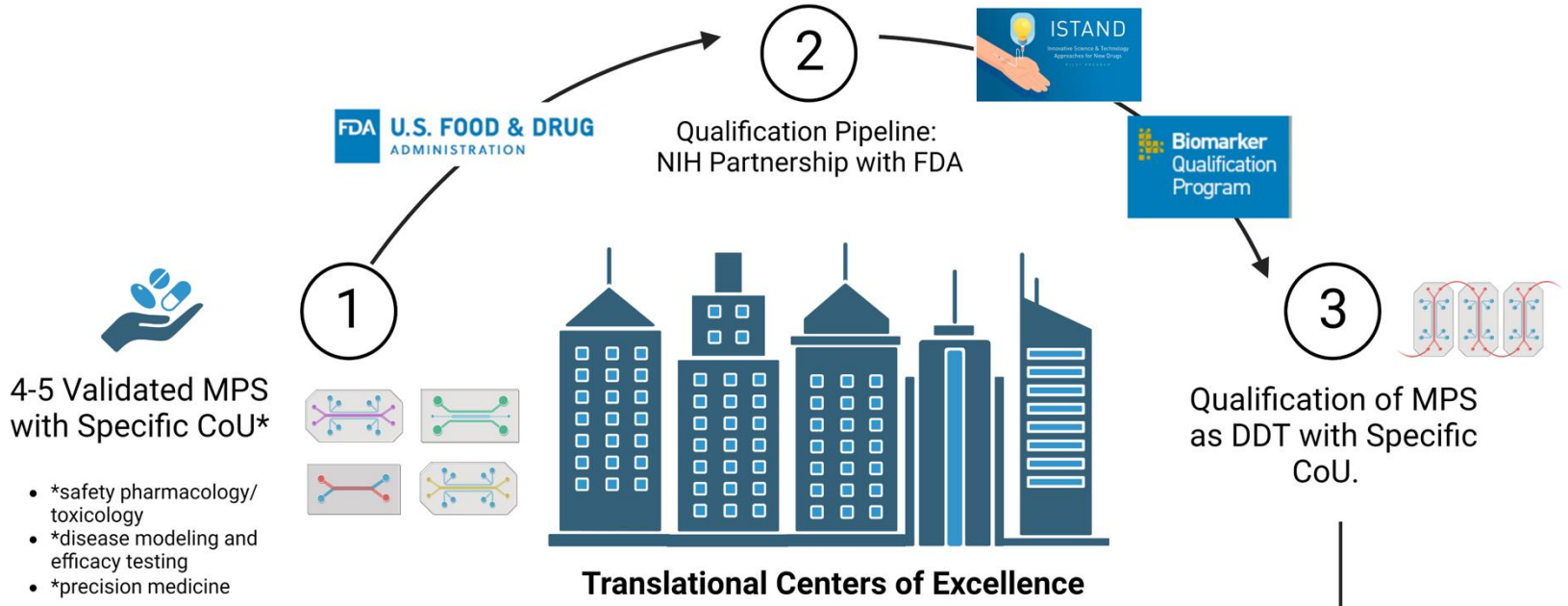


Translational Centers for Microphysiological Systems (TraCe MPS)

To accelerate the translational use of MPS in drug development through **regulatory acceptance and adoption for industrial use**

Letter of Agreement between NCATS and FDA (Critical Path Institute)

Qualifying MPS as DDT that are **fit-for-purpose for industry needs** and have **specific context of use (CoU) that will meet regulatory qualification**



- Mt. Sinai University and University of Washington
- The University of Texas Medical Branch at Galveston, Texas Engineering Experiment Station, and Texas A&M AgriLife
- University of Pittsburgh
- University of Rochester and Duke University

TraCe MPS Centers started in Spring of 2024

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DDT Qualification Submissions to FDA

- FDA DDT qualification programs for **1) biomarkers, 2) clinical outcome assessments, and 3) animal models**
  - **On average, it takes 6 -10 years for a DDT to be qualified (***Ther Innov Regul Sci* **59**, 871–881 (2025))

- **Innovative Science and Technology Approaches for New Drugs (ISTAND) Program** - Launched by FDA in 2020 as a pilot program for **NAMs**; became a permanent program 7/31/2025; intended for NAMs
  - **Use of tissue chips (i.e., microphysiological systems) to assess safety or efficacy questions**
  - **Use of artificial intelligence (AI)-based algorithms to evaluate patients, develop novel endpoints, or inform study design**
  - **Use of novel digital health technologies (e.g., wearables) for patient assessment**

ISTAND Submissions for All NAMs

https://force-dsc.my.site.com/ddt/s/

Total Number of Projects in Development	**10**
Number of Letters of Intent (LOIs) Accepted*	**9**
Number of Qualification Plans (QPs) Accepted*	**1**
Number of Newly Qualified DDTs (past 12 months)	**0**
Total Number of Qualified DDTs To Date	**0**

*As of June 30, 2025

Translational Centers for MPS ISTAND Submissions

Project #	Project Name	Submission Stage/Status
DDT-IST-000034	Liver acinus MPS (LAMPS) for determining drug candidate dosing in clinical trials of liver disease	2025-06-23– LOI Accept and QP invited 2025-02-20 – LOI Reviewable 2025-01-09 – LOI Submission
DDT-IST-00045	• Human Kidney Chip for Assessment of Relative Nephrotoxicity	• 2025-07-03 - LOI Reviewable • 2025-06-24 - LOI Submission
DDT-IST-00047	• Human chorio-decidual interface organ on chip for derisking positive rodent DART studies for new modality investigational new drug candidates	• 2025-07-15 – LOI Reviewable • 2025-07-07 – LOI Submission
DDT-IST-00044	• Liver acinus MPS (LAMPS) for hepatoxicity	• 2025-07-03 – LOI Reviewable • 2025-06-19 – LOI Submission
2 LOIs		LOI – pending submissions

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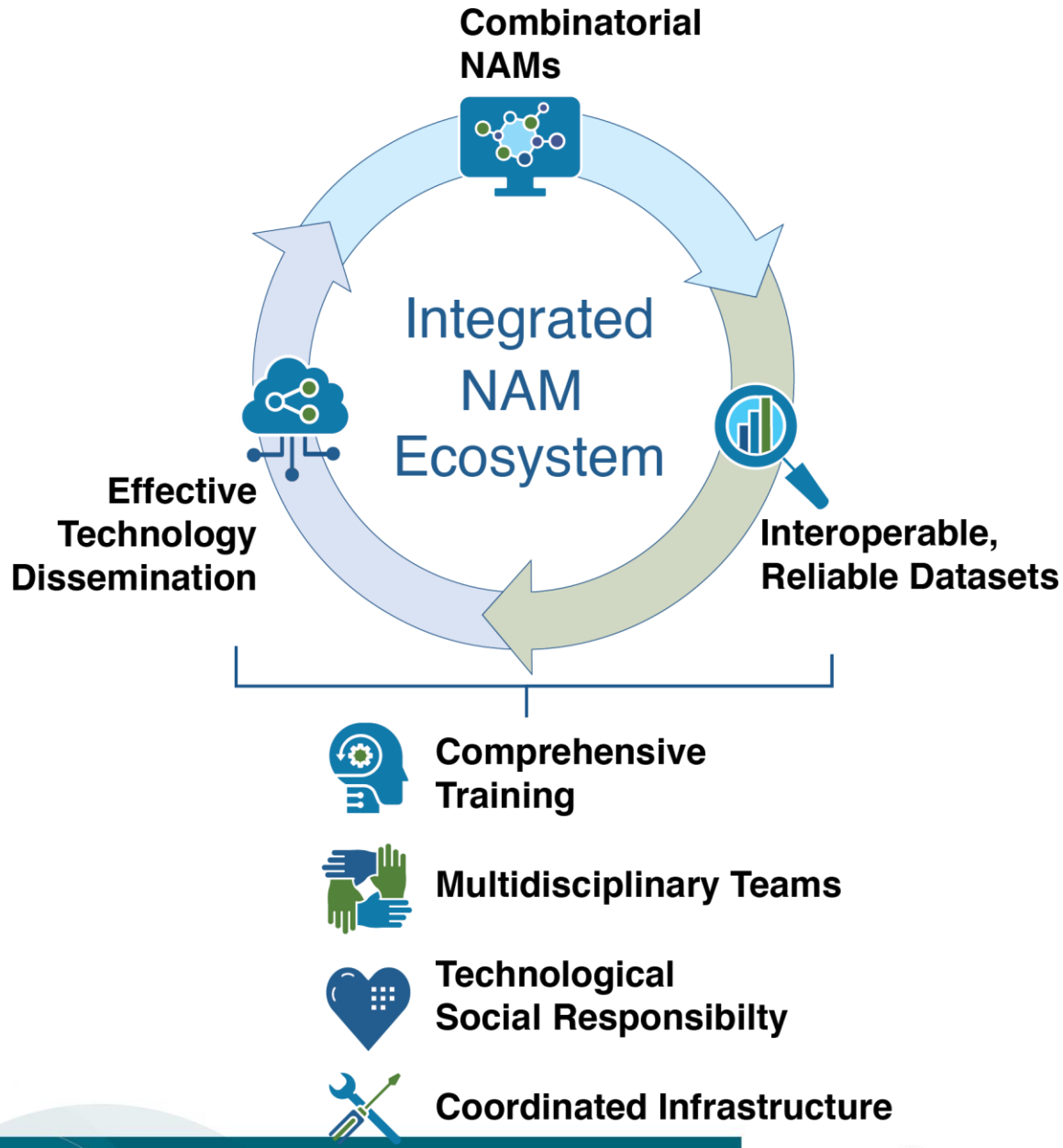
NIH VISION OF NAMs

An integrated ecosystem to catalyze scientific discovery

- *Report to Congress on NIH Investments on NAMs, March 2022*
- *NIH Advisory Committee to the Director, December 2023*
- *Implemented through Complement-ARIE program*



NIH-FDA Coordinated Efforts to Advance Goals of Complement-ARIE

- The NIH and FDA have established an MOU to collaborate and coordinate efforts that will promote and accelerate the translational use of NAMs. Efforts include:
  - Providing **regulatory expertise** (FDA) to help support, refine, and advance projects with regulatory potential within Complement-ARIE
  - Holding regular joint **NIH-FDA workshops and meetings** to ensure progress in goals and to develop new policies and initiatives as needed.
  - Engaging **in activities to promote development, adoption, and uptake of NAMs** that may have applicability for FDA regulatory use.
- A similar MOU has also been established with the EPA.

Complement-ARIE: Program Components

- ***Technology Development Centers (TDC)***– support the development of NAMs to fill in areas of greatest needs and scientific priorities, with emphasis on increased biological complexity and human relevance through innovative combinatorial approaches.
- ***NAMs Data Hub & Coordinating Center (NDHCC)***– create integrated data structures, including standards for model credibility, improve FAIRness (Findable, Accessible, Interoperable, and Reusable) of NAM-generated data, and searchable NAMs repository.
- ***Validation and Qualification Network (VQN) –*** establish framework for NAMs regulatory, industrial and community adoption and Implementation by establishing common data elements and standardized reporting and apply stakeholder-generated validation/qualification criteria through public-private partnerships.
- ***Community Engagement and Training (CET)*** *– promote the development of next-generation biomedical research workforce with the skills to build and utilize new NAMs, actively work with the stakeholder communities, and address societal and ethical considerations.*
- ***Prize Competitions*** *- Out-of-the-box solutions towards development, regulatory acceptance and widespread use of human-based NAMs to advance human relevance in biomedical research. 1)* ***Ideation Stage*** *($1M prize - 20 winners); 2)* ***Reduction to Practice*** *($7M prize – current solicitation for innovative solutions)*

https://commonfund.nih.gov/complementarie/challengewinnersummaries

NIH $400,000,000 over 10 years plus private sector funding

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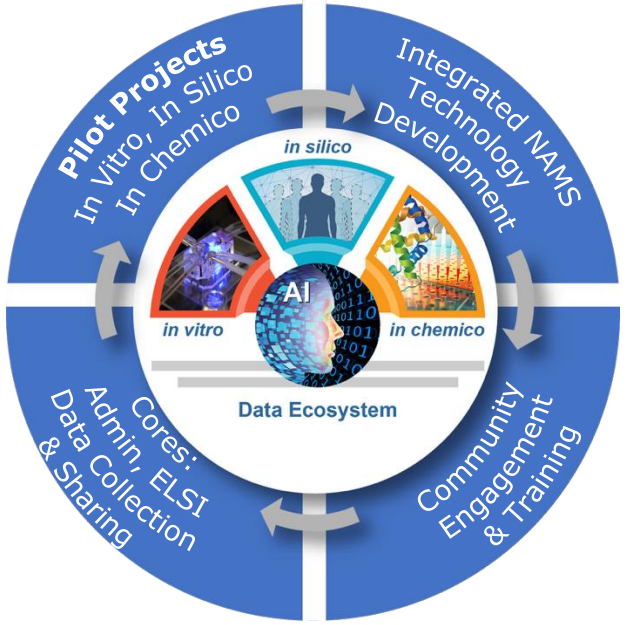
Complement-ARIE TDC Structure

- The Comprehensive NAMs TDCs is dedicated to the development of combinatorial NAMs, with an emphasis on human relevance and increased biological complexity.
- Develop mature combinatorial NAMs will be transferred to the VQN for validation and/or qualification as well-defined use cases.
- Focused on high priority scientific areas - chronicity (across lifespan); neuroscience; personalized health; cross-disease pathogenesis (e.g. developmental, metabolic, immune, reproductive health)
- **TDC Structure**
  - **Integrated NAMs Technology Development**
    - Major NAMs technology driver
    - Pilot projects for emerging technologies
  - **Cores**
    - Administration
    - Data Collection and Bioinformatics
    - Resources
  - **Technical Characterization and Regulatory Affairs**
  - **Training and Outreach**
    - Ethical, Legal and Social Implications (ELSI)
    - Workforce Development and Training
    - Community Engagement



6 – 7 TDCs across USA

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NAMs Data Hub & Coordinating Center

- **Consortium coordination:** Develop and maintain a comprehensive project management plan to ensure coordination of activities, timelines, and major milestones across Complement-ARIE TDCs, VQN, and other initiatives. Organize, manage, and administer Complement-ARIE consortium semi-annual meetings (both in-person and virtual). Facilitate Complement-ARIE working group meetings and ensure timely delivery of progress reports and fulfillment of other administrative requirements.
- **Data coordination:** Manage and standardize the data generated by the Complement-ARIE consortium. Develop and enforce metadata standards, data governance, and QC requirements for data submission and release.
- **Search and knowledge management:** Develop a metadata dashboard for datasets generated by Complement-ARIE including an API to retrieve discovered data. Manage the knowledge inferences and graphs derived from Complement-ARIE data. Develop searching and visualizing tools
- **Workbench:** Develop a cloud-based workbench (e.g., *All of Us*), foster community engagement and recruitment of new users.
- **Interoperability** with NIH-supported and other data ecosystems (non-NIH sources of relevant data (e.g., FDA, industry, CRO).

1 centralized and federated NHDCC

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NIH/FNIH Public-Private Partnership Structure

Validation and Qualification Network (VQN) for the Adoption and Implementation of NAMs

- PPP between government agencies, industry, NGOs, non-profits via **Foundation for NIH (FNIH)**
- Pre-competitive data and resource sharing
- Framework to accelerate the implementation and adoption of NAMs in research and regulatory contexts by **ensuring NAMs are robust, reliable, and reproducible**
- Demonstrate **biological relevance and establish confidence in NAMs** to ensure each NAM is "fit for purpose" for its intended use
- Synergize and coordinate with other global activities on NAMs
- Partners are developing work streams for 1) **Community Engagement & Training**; 2) **Data Standards & Integration**; 3) **Regulatory**
- **RFI – Rolling solicitation for potential pilot projects (use cases) for validation and/or qualification until December 31, 2025**

https://fnih.org/wp-content/uploads/2025/07/NAMs-VQN-RFI-073025.pdf

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List of Current VQN Participating Organizations

Scientific Steering Committee – one voting member from each partner organization, co-chair from each sector

Private-Sector Partners (Industry and Nonprofit)

- Alternatives Research and Development Foundation
- American Chemistry Council
- Americans for Medical Progress
- Ananda Devices Inc.
- Animal Welfare Institute
- Asymmetrex
- Charles River Laboratories
- CN Bio
- Critical Path Institute
- Draper
- Eyescreen, Inc.
- GlaxoSmithKline
- Health and Environmental Sciences Institute
- Hesperos
- Humane World for Animals
- Humane World for Animals International
- Humble Alliance L3C
- Institute for Invitro Sciences
- International Foundation for Ethical Research
- The Jackson Laboratory
- JRF Global
- Labcorp
- Novo Nordisk
- Physicians Committee for Responsible Medicine
- Precision Quantomics
- Radahaim
- Sanofi
- Spanios
- SRI International
- The 3Rs Collaborative
- Toxys
- UCB
- Unilever
- Vivodyne

Public-Sector Partners

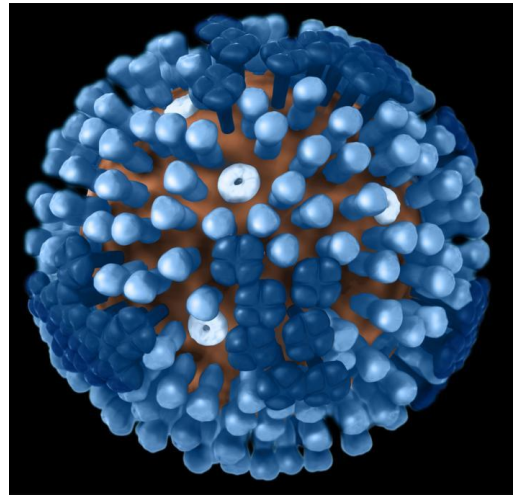
- Advanced Research Project Agency-Health
- Biomedical Advanced Research and Development Authority
- Consumer Product Safety Commission
- Department of Defense Congressionally Directed Medical Research Program
- Defense Threat Reduction Agency
- Environmental Protection Agency
- European Commission (Europe)
- Food and Drug Administration
- Interagency Coordinating Committee on the Validation of Alternative Methods
- National Aeronautics and Space Administration
- National Centre for the Replacement, Refinement, and Reduction of Animals in Research (UK)
- National Institutes of Health
- National Institute of Health Sciences (Japan)
- National Institute of Standards and Technology
- National Science Foundation
- Pharmaceuticals and Medical Devices Agency (Japan)
- US Department of Agriculture
- Veterans Administration

FNIH

Seasonal Influenza

- Influenza is a contagious respiratory illness caused by influenza viruses
- Can lead to complications (pneumonia, worsening of chronic medical conditions, such as CHF, asthma, or diabetes) and death
- Between 2010 and 2020, influenza virus infections caused 12,000 - 52,000 annual deaths in the United States
- About 8% of the population is infected with flu each season, resulting in an estimated annual economic burden (healthcare and productivity costs) of $11.2 billion
- Healthy adults may be able to infect others beginning 1 day before symptoms develop and up to 5 to 7 days after becoming sick
  - Best prevention is influenza vaccination
- Influenza among Health Care Personnel is common, and 28% – 59% of cases estimated are subclinical
  - Poses a cross-infection risk to patients

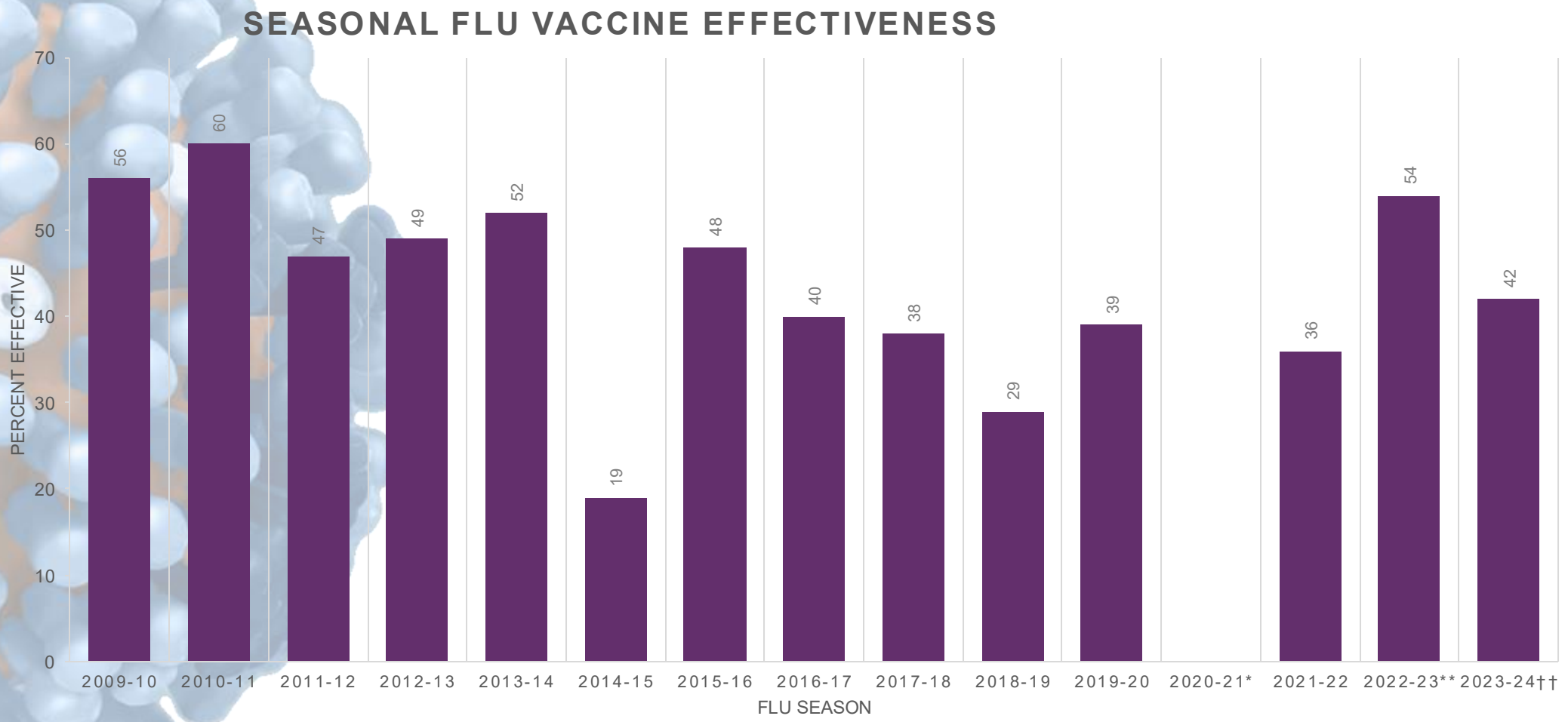
3D graphical representation of a generic Influenza virion



https://phil.cdc.gov/Details.aspx?pid=11874

1. https://www.cdc.gov/flu/keyfacts.htm
2. Wilde JA, McMillan JA, Serwint J, Butta J, O'Riordan MA, Steinhoff MC. Effectiveness of influenza vaccine in health care professionals: a randomized trial. JAMA 1999;281:908--13.

Effectiveness of Seasonal Flu Vaccines from the 2005 – 2024 Flu Seasons



Source: https://www.cdc.gov/flu/professionals/vaccination/effectiveness-studies.htm

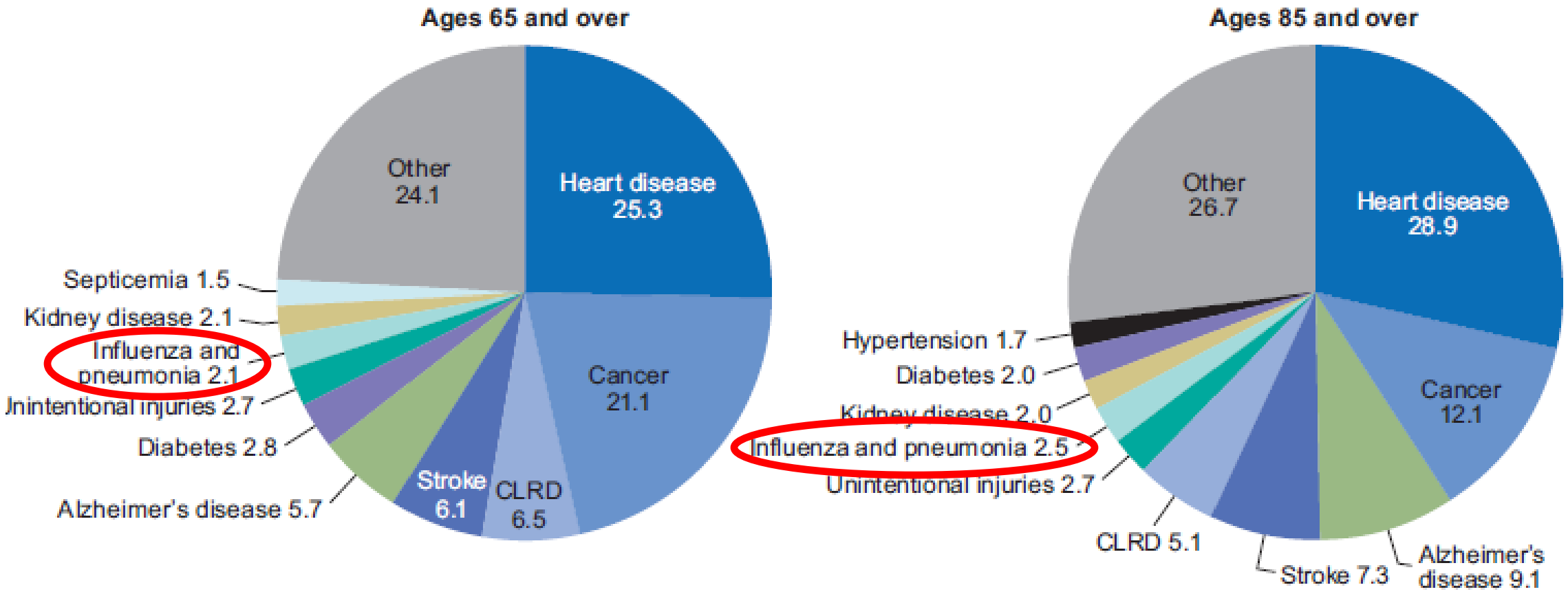
*2020-21 flu vaccine effectiveness was not estimated due to low flu virus circulation during the 2020-2021 flu season.
**In a Wisconsin study among patients aged 6 months to 64 years, VE was 54% against medically attended outpatient acute respiratory illness (ARI) associated with laboratory-confirmed influenza A.
†† VE estimates for 2022-2023 flu season are preliminary.

Impact of Seasonal Influenza in Adults ≥ 65 Years

- 54 – 70% of seasonal flu-related hospitalizations have occurred in people ≥ 65 years
- Risk is greatest in the oldest age group (≥ 85 years)
  - 16 times more likely than persons 65 – 84 years
- 70 – 85% of seasonal flu-related deaths have occurred in people ≥ 65 years
- Case fatality rates in long-term care facilities (LTCF) from influenza complications as high as 55%

1. https://www.cdc.gov/flu/about/disease/65over.htm
2. https://www.cdc.gov/mmwr/preview/mmwrhtml/rr59e0729a1.htm
3. Nace DA, Drinka P, Mann J, Poland GA. LTC Information Series: Immunization in the Long-Term Care Setting. 2nd ed. Columbia, MD: American Medical Directors Association; 2010.
4. Morens DM, Rash VM. Lessons from a nursing home outbreak of influenza A. Infect control Hosp Epidemiol. 1995; 16(5):275-80.

Top Ten Leading Causes of Death in the United States for Adults ≥ 65 Years and ≥ 85 Years (2016)



https://www.cdc.gov/nchs/data/nvsr/nvsr67/nvsr67_06.pdf

Why Are There Different Flu Vaccines?

- The influenza virus mutates continuously. The most common strains of the influenza virus around the world change 80-90% year to year.
- Flu is most common during the cooler months. Therefore, in the Northern Hemisphere, the flu season is generally October - March, while in the Southern Hemisphere, the flu season is generally April - September.
- About 60% of the time for a given season, the strains most common in the Northern Hemisphere are not the same as what started out in the Southern Hemisphere.
- Flu vaccines are based upon the best estimate of which strains will be most common during each season. When the strains in the vaccine match the circulating strains, the flu vaccine is about 60% effective, but if one or more strains is mismatched in the vaccine, then the efficacy goes down.

The Case for a Broadly Protective Influenza Vaccine

- Current vaccines are reformulated annually and elicit strain-specific responses
- Previous infection can bias the quality of strain-specific responses later in life
- Humans are repeatedly exposed to a variety of flu drift and shift variants through immunization or infection. Although there are some conserved regions, primarily in the hemagglutinin (HA) stalk domain, the immunodominant Ab response is focused on the HA head; this immune pressure drives antigenic drift
- There is an urgent need to develop a universal flu vaccine that is robust, persistent, broadly cross-reactive, and effective in the majority of the population
- There are no vaccines that stimulate broadly neutralizing antibodies (Abs) are approved for clinical use

Live Attenuated and Inactivated Flu Vaccines Produce Differing Immune Responses

- IIV (Inactivated Influenza Vaccine):
  - Administered via injection.
  - Primarily induces antibody (IgG) responses in the blood.
  - May have limited capacity to induce mucosal IgA or T-cell responses.
  - Generally considered safe and effective for most individuals.
  - In some studies, has shown higher effectiveness against certain strains like H1N1pdm09 in children compared to LAIV.

- LAIV (Live Attenuated Influenza Vaccine):
  - Administered intranasally.
  - Induces a broader immune response, including mucosal immunity (IgA) and T-cell responses (including CD4+, CD8+, and γδ T cells).
  - May better mimic natural infection.
  - Has shown superior efficacy in some studies, particularly in children.
  - Effectiveness has been variable and sometimes lower than IIV in certain seasons, particularly against H1N1pdm09.
  - Not recommended for all individuals, including those with certain health conditions like asthma.

MPS for Development and Assessment of Flu Vaccine

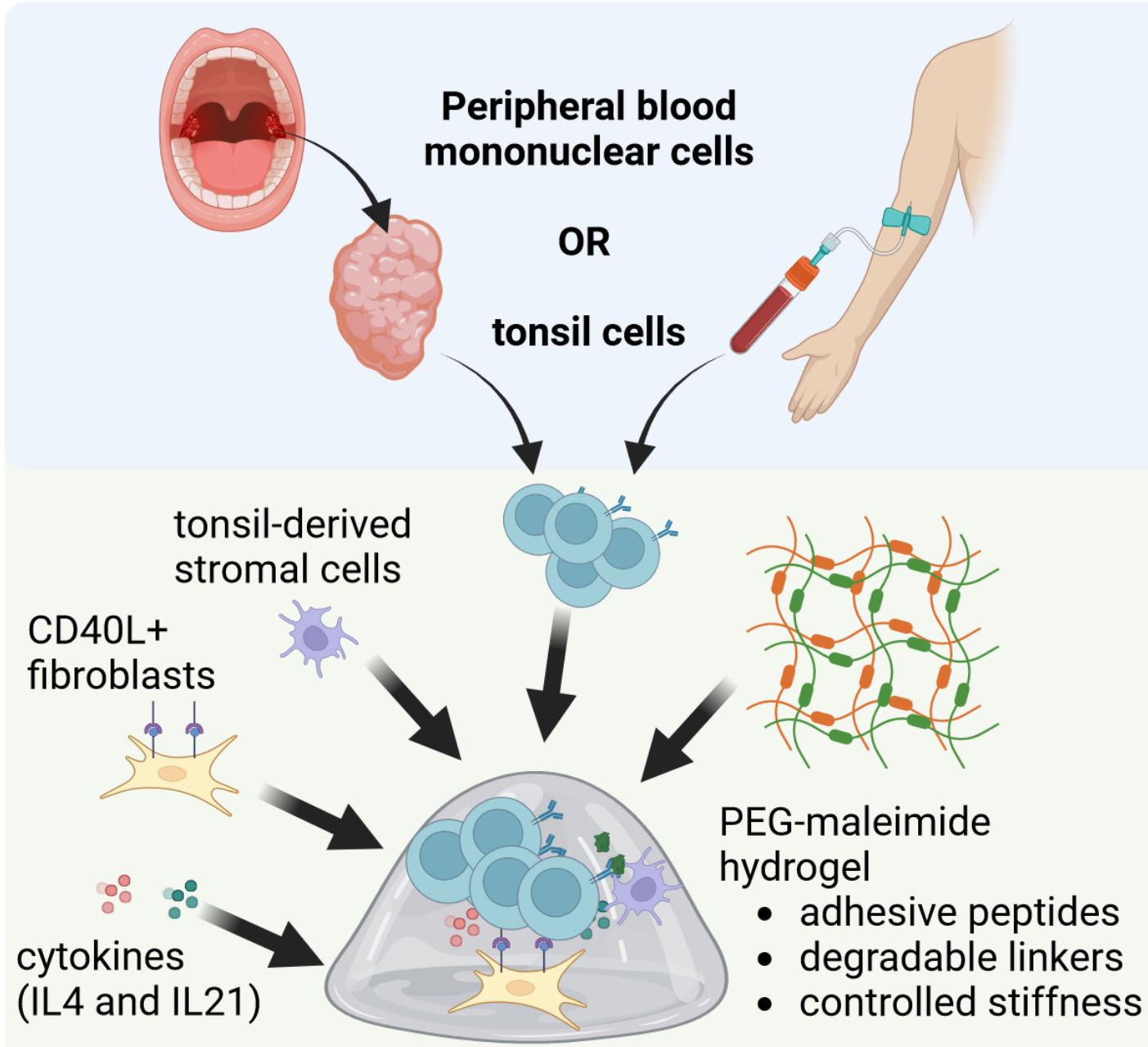
- Most human influenza studies have been limited to peripheral blood sampling, even though the critical cellular decisions that lead to productive adaptive immune responses occur within lymphoid tissues
- MPS *in vitro* organoid platform derived from primary human tonsil tissues and consist of both lymphoid and mucosal tissues.
  - Tonsil tissues are accessible from otherwise-healthy patients undergoing tonsillectomy for hypertrophy or obstructive sleep apnea.
  - Can readily represent demographically diverse populations and cover the full human age span; males and females are also represented at similar proportions. Hence able to capture host-mediated inter-individual immune variation related to patient age, sex, and immune history (such as immune-naïve vs immune-senescent status).
  - Immune organoids derived from tonsils accurately model human germinal center responses, specific antibody secretion, and T cell activation in response to influenza antigens. They contain immune cells in addition to non-hematopoietic (stromal, mesenchymal, epithelial) cells.
  - Readouts can include antigen-specific T cell expansion, B cell differentiation, specific antibody secretion, receptor repertoire analysis, and sensitivity to manipulations including adjuvant stimulation and cell depletion experiments.
  - Possible to evaluate magnitude and quality of specific antibody responses from various influenza Ag modalities and with immune modulators, such as adjuvants and TLR agonists. Ag/adjuvant combinations can be tested on high throughput system.

in vitro Lymph Nodes-on-chip

Spatial Configuration	Lymph Node Scaffold Material	ECM Material	Dynamic Condition	Fluid Flow Rate	Cellular Components	Cell Sources	Cell Culture Time	Immune System Function	Reference
A “Y” type fluidic channel	PDMS	Fibronectin	Syringe pump	0.2 mL/min (chemokine)	T cell (activation)	Human Blood	20 min	Human T cells in response to single and competing gradients of chemokine CCL19 and CXCL12.	Lin et al. (2006)
Two layers of PDMS: top contains the chemotaxis chamber, and bottom includes the T cell compartment	PDMS	Hydrogel	Syringe pump	0.4–0.5 µL/min	MUTZ-3: Human dendritic cell line	Human Blood	2 h	Mature DCs subjected to gradient effect by the chemokine CCL19 and collected in T cell compartments to induce T cell activation	Mitra et al. (2013)
Two PDMS layers: upper layer flow channels, and lower layer flow-free chambers	PDMS (0.4 µm PC membrane filter)	Fibronectin			Jurkat: Human T cell line	Cell line	30 min	Chemotaxis of Jurkat cells governed by the CXCL12 gradient and the average CXCL12 concentration	Sonmez et al. (2020)
Multi-chamber bioreactor, separated by circularly distributed microcolumns: 1) subcapsular sinus, 2) reticular ductal structure, and 3) B follicle and paracortex.	PDMS (Hydrogel microcolumns)	Hydrogel (Type I collagen)	Micropump		EB1: Human B cell line THP-1:Human Dendritic cell line Jurkat: Human T cell line	Cell lines	72 h	Long-term culture and in situ viability testing of Sertoli cells and the flow pattern of lymphatic fluid was replicated	Shanti et al. (2020)
Multi-chamber bioreactor, separated by circularly distributed microcolumns: 1) subcapsular sinus, 2) reticular ductal structure, and 3) B follicle and paracortex.	PDMS (Hydrogel microcolumns)	Hydrogel (Type I collagen)	Microfluidic pump S	3 µL/min	Raji B: Human B cell line Jurkat: Human T cell line	Cell lines		Tested the effect of the immunomodulatory drug hydroxychloroquine (HCQ) on cells	Hallfors et al. (2021)
Two channels divided by PDMS membrane; lower channel consists of T, B lymphocytes, and hydrogels, and upper channel is continuously perfused with medium	PDMS	Hydrogel	Peristaltic pumps or Automated Zoe Organ Chip instruments	60 µL/h	T cell and B cell	Human Blood	>9 d	Mimic germinal center formation, class switching, and Ab production. Antigen-specific Ab produced by Fluzone influenza vaccine for three different strains and the H5N1 pandemic influenza antigen inoculated LF chip formulated with the oil-in-water adjuvant SVE of squalene.	Goyal et al. (2022)
A central channel with an extension in the center of the main channel in which a collagen sponge is mounted, inside which cell spheroids are placed	PDMS	Collagen sponge	Micropump	0.65 mL/h	4T1 breast cancer spheroids Jurkat T cell line	Cell lines		Effect of contrast/drug vehicle size on the penetration and accumulation of particles in 3D spheroids simulating secondary tumors with lymphadenopathy	German et al. (2023)

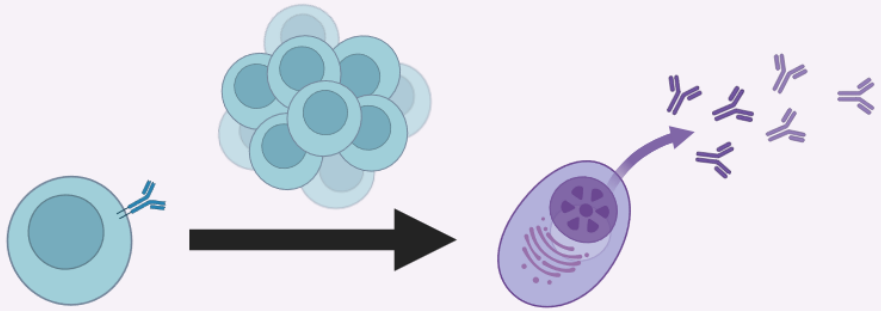
Examples of MPS Developed for Immunity Studies

Conditions	Tissues/Organs	Features	References
Influenza	Tonsil organoids	recapitulate various aspects of the adaptive immune response, including B and T cell differentiation, expansion of antigen-specific B and T cells, affinity maturation, and antibody secretion	*Nat Med. 2021;27:125-135 (Lisa Wagar's lab UC Irvine)*
Influenza, COPD	PBMCs	primary human blood B- and T-lymphocytes autonomously assemble into ectopic LFs; production of anti-HA IgG and secretion of cytokines	*Adv. Sci. 2022, 9, 210324 (Don Ingber's lab Wyss Institute)*
staphylococcal enterotoxin B	Tonsil slices on microfluidic chip to model early T and B cell interactions at the extrafollicular border	B and T cell cluster formation, plasmablast differentiation, and antibody production	*bioRxiv preprint doi: https://doi.org/10.1101/2025.01.12.632545 (Rebecca Pompano's Lab U Virginia)*
Influenza	Tonsil aggregates on a centrifugal microfluidics-based organ-on-chip with vasculature-like perfusion	long-term culture of lymphoid tissue and raised antigen-specific antibody responses against influenza vaccines even after four weeks on-chip	*bioRxiv preprint doi: https://doi.org/10.1101/2025.01.14.632762 (Peter Loskil's lab U Tubingen)*



Outputs

- B cell activation, differentiation into antibody-secreting cells



- Antibody production
- Class switching
- Somatic hypermutation
- Light and dark zones
- Spontaneous and antigen-specific responses

Applications

- Drug and immunotherapy testing
- Patient-specific models
- Incorporation with microfluidic control

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